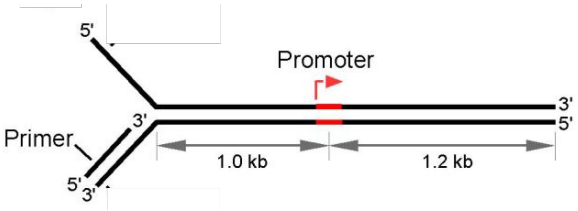
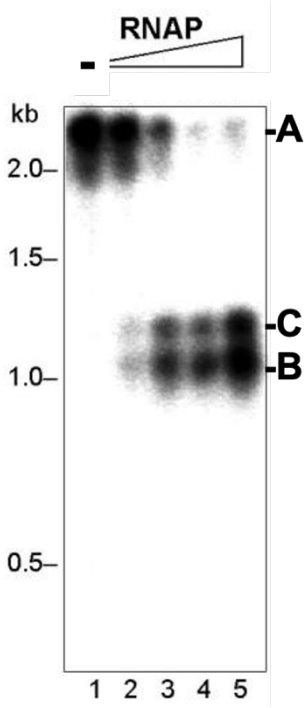
Practice Question 1.

This experiment involves a 2.2kb long DNA shown below, which contains one strand annealed to a DNA primer, followed by a double stranded region containing a promoter sequence for a bacterial RNA polymerase (RNAP). The promoter is located ~1.0kb from the primer. A bacterial replisome is added to this substrate in the presence of radiolabeled dNTPs (Lane 1). In lanes 2-5, an increasing amount of RNAP is added to the reaction, in conditions such that the RNAP synthesizes a 20 nucleotide long RNA from the promoter but is then stalled on the DNA after synthesis of the 20 nt long RNA. Reaction products are fractionated on a gel and a size marker is included. Only radiolabeled nucleic acids are detected.



IMPORTANT: identify what is radiolabeled.

A – Describe how the presence of RNAP in the reaction affects the production of the species labeled "A". What biochemical event led to the production of the species labeled "A"?



* Species A: >2.0 kb in length.

→ Increasing amounts of RNAP decreases the amount of species A.

→ Complete DNA replication by the DNA polymerase results in production of species A.

B –Describe how the presence of RNAP in the reaction affects the production of the species labeled "B". What biochemical event led to the production of "B"?

* Species B: ~1 kb in length.

→ B is not detected without RNAP. The amount of B steadily increases as the amount of RNAP is increased.

→ Based on its size, B corresponds to DNA synthesized by the DNA polymerase until it reaches the stalled RNAP, which blocks further replication.

C – Describe how the presence of RNAP in the reaction affects the production of the species labeled as "C". What biochemical event led to the production of "C"?

* Species C: ~1.2kb in length.

→ C is not detected without RNAP. The amount of C steadily increases as the amount of RNAP is increased.

→ Based on its size, C corresponds to DNA synthesized from the site where RNAP is stalled to the end of the DNA. The DNA polymerase previously blocked by the stalled RNAP was able to resume replication using the RNA that was synthesized by RNAP as a primer.

Practice Question 2.
This table includes a description of the major activities/properties of three eukaryotic DNA polymerases (DNAP).

	Pol α	Pol δ	Pol ε
Activities	Polymerase	Polymerase 3′-5′ exonuclease	Polymerase 3′-5′ exonuclease
Fidelity	$10^{-3} - 10^{-4}$	$10^{-4} - 10^{-6}$	$10^{-5} - 10^{-6}$
Processivity	low	low	high
PCNA stimulation of processivity	–	+++	+

A – Which of these DNAP would make the most mistakes? 2pts.

Pol α (Pol α has the lowest fidelity)

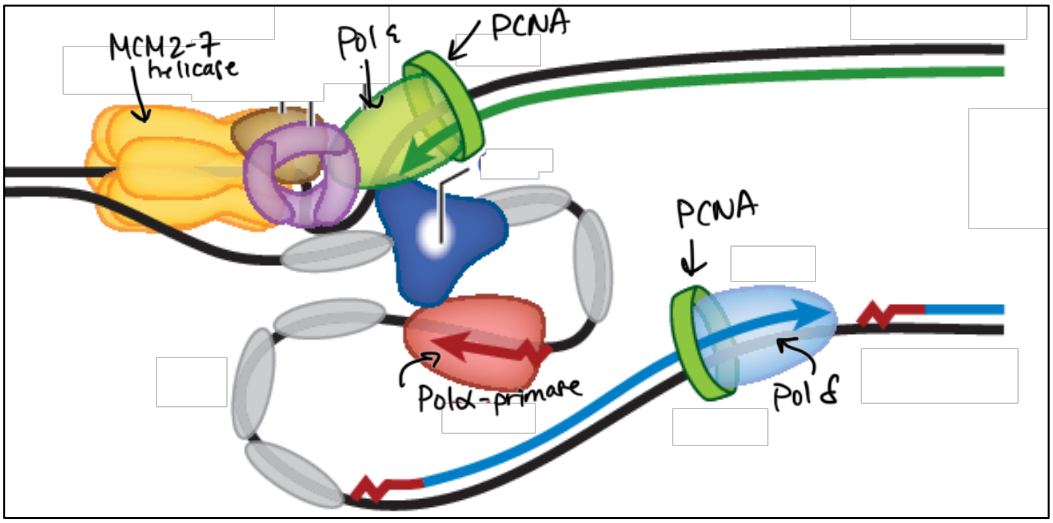
B – Which of these DNAP lacks proofreading and why? Based on the data provided above and on what you know about the function of this DNAP, explain why this DNAP does not need proofreading. 4pts.

Pol α lacks proofreading activity. (it does not have 3'→5' exonuclease activity). Pol α does not need proofreading capability because it only shortly extends RNA primers. The probability of making a mistake in such a short segment of DNA is low.

C – Provide an explanation (not just a description) for the differences listed between Pol.δ and Polε in terms of processivity and stimulation of processivity by PCNA.4pts.

Pol ε is more processive than Pol δ, but processivity is not highly stimulated compared to δ.
Why? Pol ε has a P-domain which wraps around the template-primer duplex and confers high processivity without PCNA (so PCNA won't increase processivity by much).

D – On the figure below, indicate the positions of:
1: All three DNA polymerases listed above.
2: All PCNA molecules
3: The helicase involved in unwinding the DNA. **Include the specific name of that helicase on the figure**. 4pts.



Practice Question 3.

Do the following statements apply to DNA Replication in Bacteria, Eukaryotes, Both Bacteria and Eukaryotes, or Neither Bacteria nor Eukaryotes?
Write "Bacteria", "Eukaryotes", "Both", or "Neither" as appropriate in the boxes below (1.5 pts each).

Neither	Sliding clamps unwind DNA at the replication fork.
Bacteria	Replication fork polymerases are physically linked together by a flexible protein.
Eukaryotes	Primase is fused to a polymerase that extends upon primers with short DNAs.
Both	Primers can be removed by a nuclease that acts on RNA:DNA hybrids.
Eukaryotes	Telomerase is required to prevent the progressive shortening of the genome.
Neither	DNA strands can be synthesized by DNA polymerases in the absence of a primer.
Neither	DNA Polymerase I synthesizes both the leading and lagging strands.
Eukaryotes	Nucleosomes are reassembled behind the replication fork.